PO-CON1480E

SHIMADZU
Excellence in Science

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

ASMS 2014 MP345

Stéphane MOREAU[1] and Mikaël LEVI[2]
[1] Shimadzu Europe, Albert-Hahn Strasse 6-10, Duisburg, Germany
[2] Shimadzu France SAS, Le Luzard 2, Boulevard Salvador Allende, 77448 Marne la Vallée Cedex 2, France

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Introduction

Mycotoxins are toxic metabolites produced by fungal molds on food crops. For consumer food safety, quality control of food and beverages has to assay such contaminants. Depending on the potency of the mycotoxin and the use of the food, the maximum allowed level is defined by legislation. Baby food is particularly critical. For example, European Commission has fixed the maximum level of Aflatoxin B1 and M1 to 0.1 and 0.025 µg/kg, respectively, in baby food or milk.

Therefore, a sensitive method to assay mycotoxins in complex matrices is mandatory. In order to ensure productivity of laboratory performing such assays, a unique rapid method able to measure as much mycotoxins as possible independently of the sample origin is also needed. In this study, we tested three kind of samples: baby milk powder, milk thickening cereals (flour, rice and tapioca) and a vegetable puree mixed with cereals.

Materials and Methods

Sample preparation

Sample preparation was performed by homogenization followed by solid phase extraction using specific cartridges (Isolute® Myco, Biotage, Sweden) covering a large spectrum of mycotoxins.
Sample (5g) was mixed with 20 mL of water/acetonitrile (1/1 v/v), sonicated for 5 min and agitated for 30 min at room temperature. After centrifugation at 3000 g for 10 min, the supernatant was diluted with water (1/4 v/v). Columns (60mg/3 mL) were conditioned with 2 mL of acetonitrile then 2 mL of water. 3 mL of the diluted supernatant were loaded at the lowest possible flow rate.

Then column was washed with 3 mL of water followed by 3 mL of water/acetonitrile (9/1 v/v). After drying, compounds were successively eluted with 2 mL of acetonitrile with 0.1% of formic acid and 2 mL of methanol.
The eluate was evaporated under nitrogen flow at 35 °C until complete drying (Turbovap, Biotage, Sweden).
The sample was reconstituted in 150 µL of a mixture of water/methanol/acetonitrile 80/10/10 v/v with 0.1% of formic acid.

LC-MS/MS analysis

Extracts were analysed on a Nexera X2 (Shimadzu, Japan) UHPLC system and coupled to a triple quadrupole mass spectrometer (LCMS-8050, Shimadzu, Japan). Analysis was carried out using selected reaction monitoring acquiring 2 transitions for each compound.

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Table 1 – LC conditions

Analytical column	: Shimadzu GLC Mastro™ C18 150x2.1 mm 3µm
Mobile phase	: A = Water 2mM ammonium acetate and 0.5% acetic acid B = Methanol/Isopropanol 1/1 + 2mM ammonium acetate and 0.5% acetic acid
Gradient	: 2%B (0.0min), 10%B (0.01min), 55%B (3.0min), 80%B (7.0 -8.0min), 2%B (8.01min), Stop (11.0min)
Column temperature	: 50°C
Injection volume	: 10 µL
Flow rate	: 0.4 mL/min

Table 2 – MS/MS conditions

Ionization mode	: Heated ESI (+/-)
Temperatures	: HESI: 400°C Desolvation line: 250°C Heat block: 300°C
Gas flows	: Nebulizing gas (N2): 2 L/min Heating gas (Air): 15 L/min Drying gas (N2): 5 L/min
CID gas pressure	: 270 kPa (Ar)
Polarity switching time	: 5 ms
Pause time	: 1 ms
Dwell time	: 6 to 62 ms depending on the number of concomitant transitions to ensure a minimum of 30 points per peak in a maximum loop time of 200 ms (including pause time and polarity switching)

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Table 3 – MRM transitions

Name	Ret. Time (min)	MRM Quan	MRM Qual
15-acetyldeoxynivalenol (15ADON) [M+H]+	3.37	339 > 297.1	339 > 261
3-acetyldeoxynivalenol (3ADON) [M+H]+	3.37	339 > 231.1	339 > 231.1
Aflatoxine B1 (AFB1) [M+H]+	3.78	312.6 > 284.9	312.6 > 240.9
Aflatoxine B2 (AFB2) [M+H]+	3.57	315.1 > 259	315.1 > 286.9
Aflatoxine G1 (AFG1) [M+H]+	3.46	329.1 > 242.9	329.1 > 199.9
Aflatoxine G2 (AFG2) [M+H]+	3.26	330.9 > 244.9	330.9 > 313.1
Aflatoxine M1 (AFM1) [M+H]+	3.30	329.1 > 273	329.1 > 229
Alternariol [M-H]-	4.78	257 > 214.9	257 > 213.1
Alternariol monomethyl ether [M-H]-	5.81	271.1 > 255.9	271.1 > 228
Beauvericin (BEA) [M+H]+	8.03	784 > 244.1	784 > 262
Citrinin (CIT) [M+H]+	4.16	251.3 > 233.1	251.3 > 205.1
D5-OTA (ISTD)	5.22	409.2 > 239.1	N/A
Deepoxy-Deoxynivalenol (DOM-1) [M-H]-	3.02	279.2 > 249.3	279.2 > 178.4
Deoxynivalenol (DON) [M-CH3COO]-	2.61	355.3 > 295.2	355.3 > 265.1
Deoxynivalenol 3-Glucoside (D3G) [M+CH3COO]-	2.45	517.5 > 457.1	517.5 > 427.1
Deoxynivalenol 3-Glucoside (D3G) [M+CH3COO]-	2.45	517.5 > 457.1	517.5 > 427.1
Diacetoxyscirpenol (DAS) [M+NH4]+	1.20	384 > 283.3	384 > 343
Enniatin A (ENN A) [M+H]+	8.51	699.2 > 682.2	699.3 > 210
Enniatin A1 (ENN A1) [M+H]+	8.22	685.3 > 668.3	685.3 > 210.1
Enniatin B (ENN B) [M+H]+	7.57	657 > 640.4	657 > 195.9
Enniatin B1 (ENN B1) [M+H]+	7.92	671.2 > 654.2	671.2 > 196
Fumagillin (FUM) [M+H]+	6.16	459.2 > 131.1	459.2 > 338.7
Fumonisine B1 (FB1) [M+H]+	4.10	722.1 > 334.2	722.1 > 352.2
Fumonisine B2 (FB2) [M+H]+	4.71	706.2 > 336.3	706.2 > 318.1
Fumonisine B3	4.38	706.2 > 336.2	706.2 > 688.1
Fusarenone-X (FUS-X) [M+H]+	2.84	355.1 > 247	355.1 > 175
HT2 Toxin [M+Na]+	4.58	446.9 > 344.9	446.9 > 285
Moniliformin (MON) [M-H]-	1.16	97.2 > 40.9	N/A
Neosolaniol (NEO) [M+NH4]+	2.90	400.2 > 215	400.2 > 185
Nivalenol (NIV) [M+CH3COO]-	2.41	371.2 > 280.9	371.2 > 311.1
Ochratoxin A (OTA) [M+H]+	5.53	404.2 > 239	404.2 > 358.1
Ochratoxin B (OTB) [M+H]+	4.83	370.2 > 205.1	370.2 > 187
Patulin (PAT) [M-H]-	2.35	153 > 81.2	153 > 53
Sterigmatocystin (M+H]+	5.60	325.3 > 310	325.3 > 281.1
T2 Tetraol [M+CH3COO]-	1.64	356.8 > 297.1	356.8 > 59.1
T2 Toxin [M+NH4]+	4.94	484.2 > 215	484.2 > 305
Tentoxin [M-H]-	4.77	413.1 > 140.9	413.1 > 271.1
Tenuazonic acid (TEN) [M-H]-	4.51	196.1 > 138.8	196.1 > 112
Wortmannin (M-H)	3.95	426.9 > 384	426.9 > 282.1
Zearalanol (alpha) (ZANOL) [M-H]-	5.17	321.3 > 277.2	321.3 > 303.2
Zearalanol (beta) (ZANOL) [M-H]-	4.85	321.3 > 277.2	321.3 > 303.1
Zearalanone (ZOAN) [M-H]-	5.43	319 > 275.1	319 > 301.1
Zearalenol (alpha) (ZENOL) [M-H]-	5.25	319.2 > 275.2	319.2 > 160.1
Zearalenol (beta) (ZENOL) [M-H]-	4.94	319.2 > 275.2	319.2 > 160.1
Zearalenone (ZON) [M-H]-	5.52	316.8 > 174.9	316.8 > 131.1

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Results and discussion

Method development

LC conditions were transferred from a previously described method (Tamura et al., Poster TP-739, 61st ASMS). In particularly, the column was chosen to provide very good peak shape for chelating compounds like fumonisins thanks to its inner PEEK lining.

Small adjustments in the mobile phase and in the gradient program were made to handle more mycotoxins, especially the isobaric ones. These modifications are reported in the Table 1.

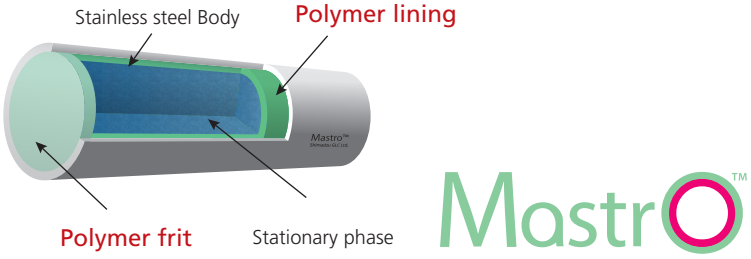

Figure 1 – Structure of the Mastro™ column

Also, autosampler rinsing conditions were kept to ensure carry-over minimisation of some difficult compounds. Electrospray parameters (gas flows and temperatures) were cautiously optimized to find the optimal combination for the most critical mycotoxins (aflatoxins). Since these parameters act in a synergistic way, a factorial design experiment is needed to find it. Manually testing all combinations in the chromatographic conditions is very time consuming. Therefore, new assistant software (Interface Setting Support) was used to generate all possible combinations and generate a rational batch analysis. Optimal combination was found in chromatographic conditions. The difference observed between optimum and default or worst parameters was of 200 and 350%, respectively.

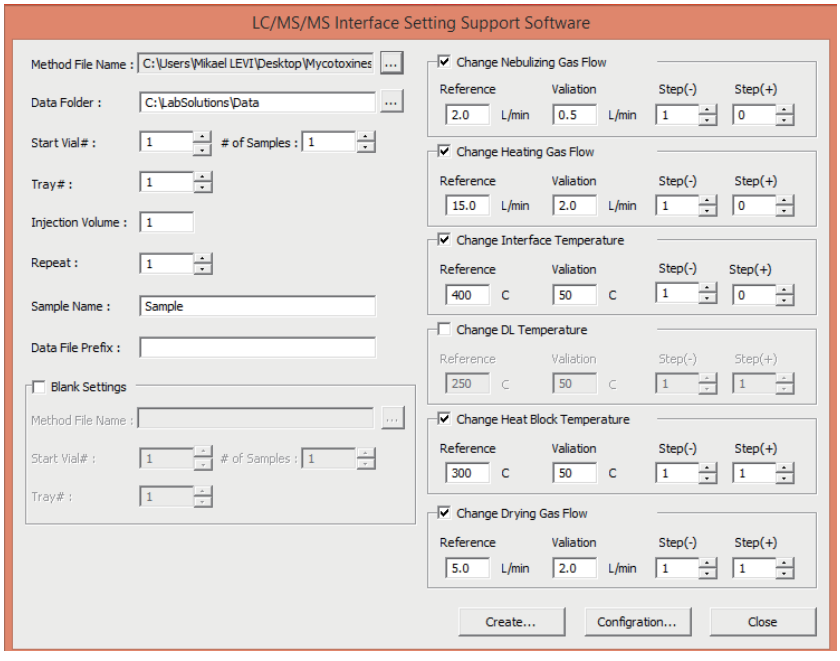

Figure 2 – Parameters selection view in the Interface Setting Support Software

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Results

Extraction and ionisation recovery for aflatoxins was measured in the three matrices by comparing peak areas of the raw sample extract to extract spiked at 50 ppb after or before extraction and to standard solution. Results in table 4 showed that the total recovery was quite acceptable to ensure accurate quantification. Results from other matrices were not significatively different.

Table 4 – Extraction and ionisation recoveries in puree

	AFB1	AFB2	AFG1	AFG2	AFM1
Extraction recovery	101%	109%	104%	114%	118%
Ionisation recovery	49%	90%	96%	106%	91%
Total recovery	49%	98%	100%	121%	108%

Repeatability was evaluated at low level for aflatoxins. Figure 3 shows an overlaid chromatogram (n=4) for aflatoxins.

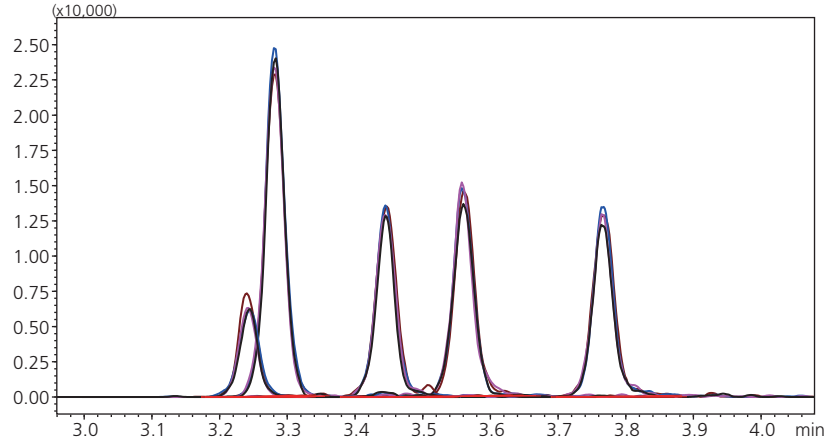

Figure 3 – Chromatogram of aflatoxins at 0.1 ppb in milk thickening cereals

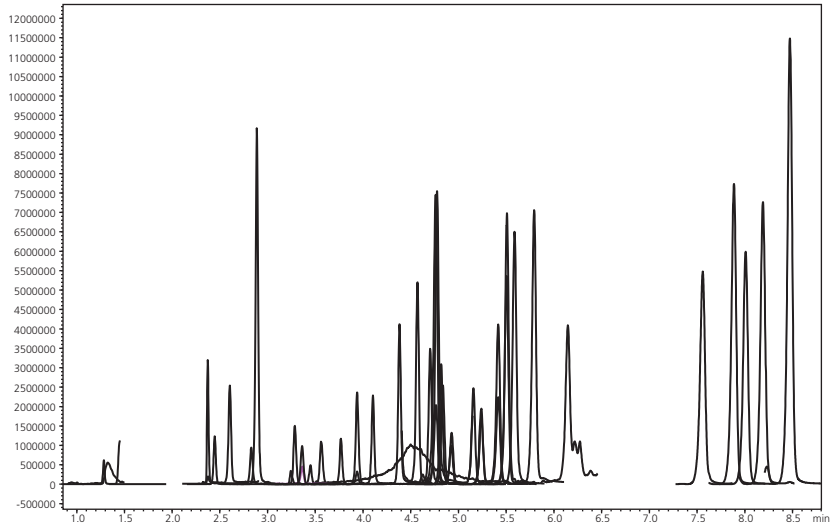

Figure 4 – Chromatogram of the 45 mycotoxins in standard at 50 ppb (2 ppb for aflatoxins and ochratoxines)

Highly sensitive and rapid simultaneous method for 45 mycotoxins in baby food samples by HPLC-MS/MS using fast polarity switching

Conclusion

- A very sensitive method for multiple mycotoxines was set up to ensure low LOQ in baby food sample,
- Thanks to high speed polarity switching, a high number of mycotoxines can be assayed using the same method in a short time,
- The extraction method demonstrate good recoveries to ensure accurate quantification.

First Edition: June, 2014

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/